

As the computational study tracks the remarkable changes in the extent of outward opening, we were able to identify the allosteric elements that are critical in mediating the impact of the perturbations on the conformational transition. Together, these findings shed new light on the Na^+ -driven transport cycle and on the dynamic details of the nature and role of allostery in the structural rearrangements associated with outward- and inward-open transitions.

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Modeling Assembly of the Tata Pore Forming Complex using an Implicit Membrane Model

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Many vital cellular processes, such as protein translocation, proton transport or molecular recognition, are mediated by self assembling membrane proteins. We have investigated the Twin-arginine translocase (TatA) complex, which forms transient pores through which proteins are translocated through the membrane. We postulated that complex formation is electrostatically driven by formation of salt bridges between amphiphilic transmembrane segments of the individual monomers and developed a structure-based model for this process[1].

We studied the formation of oligomers of different sizes by structure-based[2] MD simulations in combination with NMR constraints and a hydrophobic-slab implicit membrane model. Starting from isolated monomers, distributed far apart from each other, we observed the formation of stable TatA oligomers on the basis of the postulated interactions. The dimensions of the resulting TatA complex agreed well with experimental electron microscopy measurements[3] and the postulated interactions were confirmed by subsequent mutation studies.

[1] T. Walther, C. Gottselig, S. Grage, M. Wolf, A. Vargiu, Marco J. Klein, S. Vollmer, S. Prock, M. Hartmann, S. Afonin, E. Stockwald, H. Heinzmann, W. Wenzel, P. Ruggerone, A. Ulrich. Folding and self-assembly of the TatA translocation pore based on a novel charge zipper mechanism, Cell (accepted)

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[3] Gohlke, U. et al. The TatA component of the twin-arginine protein transport system forms channel complexes of variable diameter. Proc. Natl. Acad. Sci. U.S.A. 102, 10482-10486 (2005).

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Outward- to Inward-Facing Transition of MsbA Transporter: A Mechanistic Picture at Atomic Resolution

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MsbA is a structurally well-characterized, bacterial ATP-binding cassette (ABC) exporter. It undergoes a large-scale conformational change between the inward-facing (IF) and the outward-facing (OF) states during its transport cycle. Despite extensive experimental and computational studies on MsbA and its homologs, a unified mechanistic picture describing the OF-IF conformational transition of ABC exporters is still missing. In order to study this transition at an atomic level, we have used a novel approach based on a nonequilibrium driven scheme and performed an extensive set of molecular dynamics simulations that sample the OF-to-IF reaction-path ensemble of the *apo* MsbA in the presence of explicit solvent and membrane. using several distinct system-specific biasing protocols, we were able to steer the system along a large number of different paths in a low-dimensional holonomic coordinate space. Nonequilibrium work relations were employed to interpret the results and to determine the optimal transition path. Our results provide clear evidence that the opening of the cytoplasmic gate in the *apo* MsbA is prohibitively disfavored when the extracellular gate is open, an observation consistent with the “alternating-access mechanism”. More interestingly, we observe that the closure of the extracellular gate does not result in a stable IF conformation unless the nucleotide-binding domains (NBDs) undergo a “twisting” motion that involves a drastic change in their relative orientation. We thus propose a “door-knob mechanism” for the OF-to-IF transition of MsbA that asserts a crucial role for the “twisting” of NBDs in the opening of the cytoplasmic gate. More generally, our results call into question the simplistic models of NBD dissociation/dimerization that ignore the important role of the relative orientation between the NBDs in promoting the OF-IF conformational transitions of ABC exporters.

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Structural Transition between the Inward- and Outward-Facing States of the Glutamate Transporter in Different Lipid Environments

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Excitatory amino acid transporters (EAATs) are membrane proteins responsible for the reuptake of glutamate from the synaptic cleft in the central nervous system. Crystallographic data of a bacterial EAAT homologue, GltPh, have provided structural information for this trimeric secondary active transporter in different states. Nevertheless, in contrast to other membrane proteins, such as GPCRs, little is known about the structural or functional coupling between EAATs and their membrane environment. In this context, we investigated the effect of lipid environment on the structure and dynamics of GltPh, using all-atom molecular dynamics (MD) simulations of both the outward- and inward-facing conformations of GltPh in either POPC or POPE lipid membranes. The transition between the two states was explored with a variant of targeted MD simulations (sTMD) combining stepwise targeted motion and equilibration. Both the end conformations and the transition pathways were found to be robust to the choice of lipid bilayer type (pairwise trajectory RMSDs < 2.5 Å), implying constant contributions from the energetics of membrane deformation and hydrophobic mismatch computed with 3D-CTMD. The transition pathways connecting the end states agree substantially with the structural intermediates of a path identified recently using the method of motion planning (MP) coupled with MD (minimum RMSDs < 3 Å). Importantly, the agreement includes the prediction that the transport-trimerization domain interface changes continuously during the transition, exhibiting in the middle a significantly reduced contact area and significantly increased solvent-accessibility compared to the end states. This remarkable consistency between simple MP and the new sTMD modeling approaches indicates that the computationally economical MP calculations provide a useful initial modeling tool for major conformational transitions in membrane proteins.

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Ion-Coupling of Conformational Equilibrium and Transition in Secondary Active Transporters

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In secondary active transporters, the electrochemical potential of ions across the membrane is used to fuel the “uphill” translocation of the substrate across membranes via the alternating access mechanism. The mechanism of this crucial coupling, however, is still ambiguous, despite significant recent experimental and computational progress along structural basis and ion-binding effects. Mhp1, Benzyl-hydantoin transporter, has become a key model for the secondary active transporters sharing a similar LeuT-fold topology. In the present study, we employed molecular dynamics simulations to study the impact of Na^+ -binding on dynamics and conformational stability of Mhp1 in multiple states and transition between them. We performed microsecond equilibrium MD simulations in outward-facing (OF) states, and biased simulations with constraint of the Na^+ -binding site in the inward-facing (IF) state. The simulations suggest that Na^+ binding can stabilize the substrate-binding conformation in the OF state, but without a similar effect in its IF state, and reveal the underlying molecular mechanism in detail. Furthermore, the results of a special-protocol time-dependent biased simulation for state transition, suggest that Na^+ binding can increase the free energy barrier along the OF-IF transition. All the results suggest that ion binding can reshape the free-energy landscape of the ion/protein complex, thereby shifting the conformational preference toward a specific OF structure, which is favorable for substrate-binding. The increased substrate affinity provided by Na^+ binding will facilitate capturing the substrate from its low-concentration environment by the transporter. The results, therefore, provide a deeper and more comprehensive understanding for the ion-coupling mechanism of secondary active transporters.

Excitation-Contraction Coupling II

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Nox2-Dependent Redox Regulation of Calcium Influx in Dystrophic Skeletal Muscle

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Duchenne Muscular Dystrophy (DMD) is an X-linked, muscle-wasting disease caused by deletions in the gene that encodes dystrophin, an integral